Aspects of the biology and ecology of the Orange-Bellied Crayfish, *Euastacus mirangudjin* Coughran 2002, from northeastern New South Wales.

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ABSTRACT

The biology of *Euastacus mirangudjin*, a small freshwater crayfish from subtropical eastern Australia, is described for the first time. Long-term monitoring was undertaken at a site in Toonumbar National Park to gather data on growth, moulting, maturity and reproductive activity. To complement field observations, egg and juvenile development was further studied in laboratory aquaria. The orange-bellied crayfish reaches less than 40 mm carapace length, with female maturity occurring near 30 mm. Animals do not respond to baits, and appear to spend most of their time within their burrows. The species is an Autumn-Spring brooder with a distinctly low fecundity and large, ovoid eggs (3.0 x 3.8 mm), and females do not appear to routinely moult prior to spawning. At some sites, the species co-occurs with the much larger and spinier *E. sulcatus*. A high incidence of regenerate chelipeds, missing limbs and other wounds was recorded. *Euastacus mirangudjin* hosts a small, unpigmented Temnocephalan flatworm. The biology and ecology of the species is discussed with reference to other species in the genus *Euastacus*.

Key words: burrow systems, Clarence river, crustacean, Decapoda, freshwater crayfish, growth, highland headwaters, Parastacidae, Richmond River, reproductive biology, subtropical rainforest.

Introduction

Euastacus mirangudjin (Figure 1) has recently been described and at the time was known only from the type locality (Coughran 2002). Consequently, fewer than five specimens were examined in the original description, as the species was present in very low numbers (Coughran 2002). Apart from minor notes in the original description regarding a berried female the biology of this species is undocumented. Morphologically and ecologically, this species belongs to an identifiable subset of the genus *Euastacus*, characterized by their small size, poor spination and a comparatively northern biogeography (Coughran 2008). A conservation assessment for several of these 'poorly-spinose' species identified *E. mirangudjin* as threatened, and documented the limited distribution and habitat information available for the species (Coughran 2007). The aim of this paper is to examine the basic biology and ecology of *Euastacus mirangudjin* (including population structure, behaviour, reproduction, growth and moulting), and identify key research gaps on this and other poorly-spinose species in the region.

Figure 1. The Orange-bellied Crayfish, *Euastacus mirangudjin* (Coughran 2002). Photo, R. B. McCormack.

Methods

Methods here follow those outlined in an earlier paper (Coughran 2011), and are only briefly summarised here.

Habitat

Habitat data were obtained during widespread sampling of the region. The distribution and habitat of the species have been documented broadly elsewhere (Coughran 2007), and in the present paper observations are restricted in focus to fine-scale aspects of the species' habitat (e.g. burrows, stream characteristics, water quality).

Long-term monitoring

A long-term monitoring program for *Euastacus mirangudjin* was undertaken at one site in upper Iron Pot Ck (above the falls), in Toonumbar National Park. The site commenced from a point approximately 500 m upstream of the Murray Scrub Fire Trail bridge, and extended for a further 500 m. This site was chosen as it was considered to have the most suitable substratum (and hence habitat) for crayfish. Downstream the stream was characterised by a solid bedrock substratum with limited sediment, leaf litter and cobbles and pilot sampling had revealed very few crayfish in this part of

the system. Iron pot Creek was the only known locality for *E. mirangudjin* when the biological monitoring period commenced. Monitoring of crayfish (size, sex, maturity, reproductive activity, growth) and water quality (pH, dissolved oxygen, water temperature, conductivity) was undertaken over the period August 2001 to July 2003.

Collection and marking

Crayfish were collected by hand after lifting rocks or woody debris, or by careful burrow excavation. Animals to be marked for recapture purposes were given a unique identifier by means of a tailfan clipping code (Coughran 2011). Some animals with tailfan damage, and some berried females, were excluded from marking. After marking and recording data (see below), crayfish were returned to the water at the point of capture. Animals retained for reproductive or taxonomic studies were returned to Southern Cross University in moist hessian sacks or cooled plastic containers containing some natural vegetation or leafy debris and a small amount of water. Voucher specimens were euthanased by freezing prior to being stored in 70% ethanol, either directly or after fixation in a 10% neutral buffered formalin solution for two weeks. Voucher specimens were lodged with the Australian Museum, Sydney (AM) or the Southern Cross University Collection, Lismore (SCU). The National Parks and Wildlife Service's Frog Hygiene Protocol (NSW National Parks and Wildlife Service 2001) was followed throughout the study.

Measurements and moult state

Individual crayfish were measured with vernier calipers (to the nearest 0.1 mm) for orbital carapace length (OCL), propodal length (PropL) and abdomen width (AbW) (Coughran 2011). Very small animals (<15 mm OCL) and moulting animals were not marked or measured. Animals were also checked for: the presence or absence of temnocephalan flat worms; sex (including aberrant individuals); reproductive state; injuries and wounds; missing or regenerate pereiopods; softness of carapace (i.e. recently moulted or approaching moult); and the overall cleanliness of the ventral body surface (see Coughran 2011). Growth was examined in the field during the mark-recapture study according to pre- and post-moult carapace measurements.

Reproductive studies

Females were assigned to maturity states as their gonopores progressed from calcified and lacking setal development (immature) to membraneous and setose (mature). Females with intermediate signs of development (e.g. gonopores still calcified but with an incised rim) were classed as adolescent. Fecundity was estimated visually for each female bearing a clutch of eggs or young, and four berried females were retained for development studies in laboratory aquaria at Southern Cross University, following the methods outlined for *Euastacus gumar* (Coughran 2011).

Results

Habitat and ecology

The species is known only from five highland (420-650 m) sites, all of which are in rainforest habitats within Toonumbar and Yabbra National Parks (Figure 2; see also Coughran 2007). The watercourses range from moist gullies to streams, and are located in the Richmond and Clarence river basins. The species is sympatric with *E. sulcatus* at three of the sites in Toonumbar National Park. At all sites the substrate is fine, with rock cover and woody and leafy debris. The stream bed at the long-term monitoring site contained sparse (in pools) to dense (in riffles) pebble and cobble cover. The site also had sparse to moderate boulder cover, sparse to dense leafy debris and much larger woody debris, ranging from large palm fronds to fallen trees and branches. Rocks were generally igneous in nature. The stream reached up to five metres in width in some pools, which also frequently reached depths of 1 or 2 metres. The stream flowed at most times, and most sections held some surface water for the duration of the monitoring period. The banks ranged from gentle to very steep, and the surrounding vegetation was subtropical rainforest. The site received little sunlight, and aquatic plants were absent. Exposed rocks in the stream bed, however, were commonly covered with terrestrial plants such as Rainforest Spinach, *Elatostema reticulatum*.

The species digs deep, winding burrows under debris or rock cover in the stream bed and in the stream banks. Direct burrows in the stream bed, unassociated with rocks, are uncommon. Burrows often extend onto raised shoulders and islands in the stream bed, and occasionally extend onto the forest floor immediately adjacent to the stream. Animals were difficult to catch when lifting rocks as they disappeared quickly down their burrows when disturbed. No animals could be lured from burrows with baits or baited traps, and only once during this study was an animal observed out of a burrow. Two sites were moist gullies that lacked surface water at the time of sampling. At the remaining three sites with water, the conductivity was relatively low (51-232 $\mu s.cm^{-1}$), pH ranged from 6.08 to 8.04, water temperature was mild (7-24°C) and dissolved oxygen was 6.08 mg/L or higher. All sites were well shaded by the subtropical rainforest canopy.

Size, growth, condition and moulting

One hundred and sixty-one individuals were marked at the long-term sampling site in upper Iron Pot Creek. Five (3.1%) were recaptured once during the study and marks were easily recognisable even after moulting due to a distinct warping of the regrowing integument. Throughout the study period, one animal was caught which bore a tailfan that had some likely marks, yet was unidentifiable due to tailfan damage. This animal was examined and measured, but excluded from analyses involving recapture data. In all, 196 animals (including recaptured animals and those not marked) were measured and examined at upper Iron Pot Creek. A further six animals were measured and examined in the

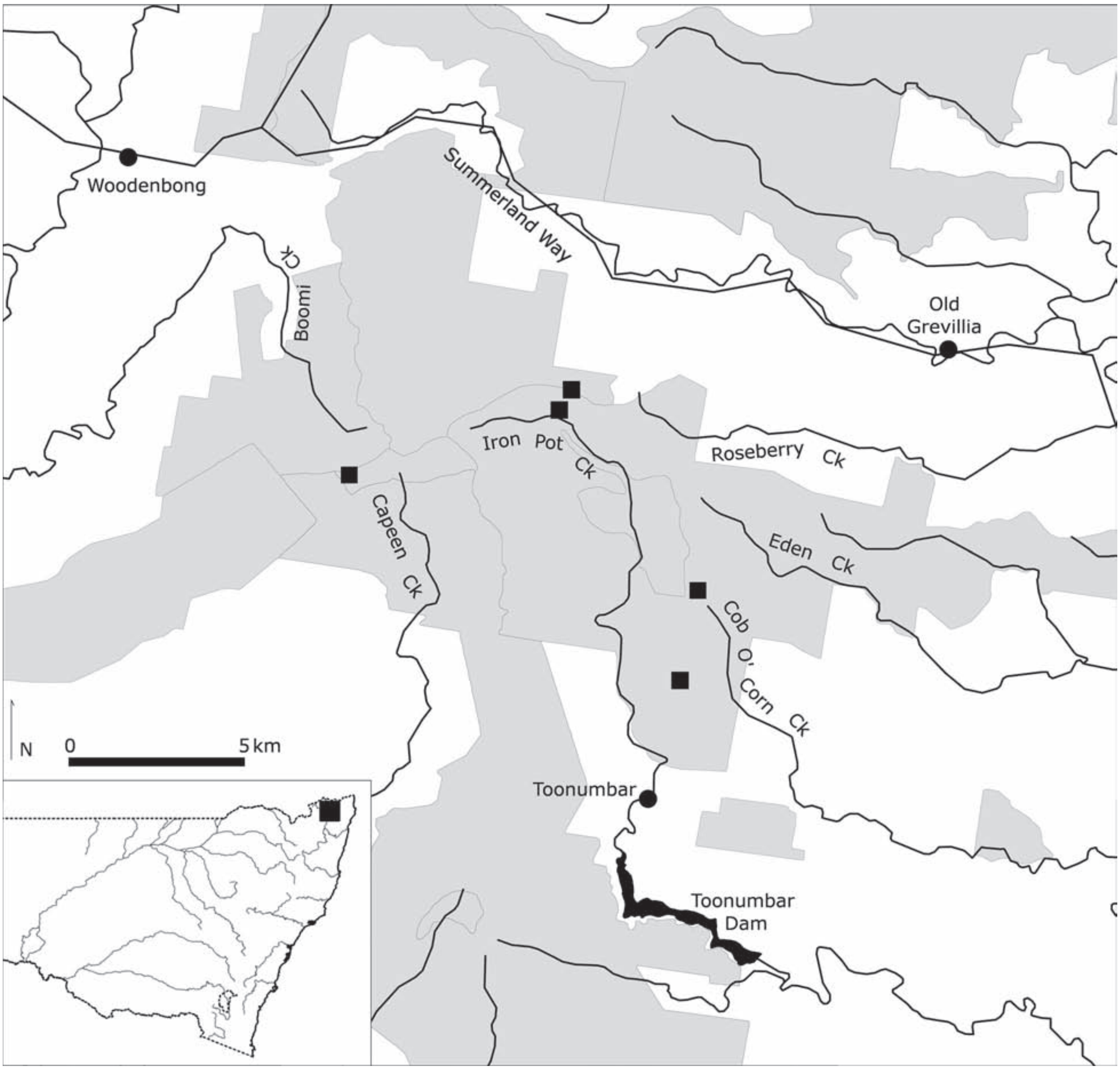

Figure 2. Distribution of *Euastacus mirangudjin.*

field from other sites in the broad distribution studies. The largest animal captured was a 38.3 mm OCL male, exceeding the previous maximum of 37.0 mm (Coughran 2002). The smallest free-living *E. mirangudjin* caught was 10.7 mm OCL. The overall male:female sex ratio for specimens >15 mm OCL was 1:1.15 (n=189), and this was not significantly different from a 1:1 ratio.

Only 50% of animals bore two normal (non-regenerate; <1 mm difference) chelipeds. Other animals were either missing a cheliped or bore only soft chela buds (10.6%), had a regenerate cheliped (>3 mm smaller than its counterpart; 26.6%), or had a slightly regenerate cheliped (1-3 mm smaller than its counterpart; 12.8%). Some animals had both chelae in a regenerative state. In addition to regenerate chelipeds, other wounds were recorded on 25.5% of animals, usually involving missing or damaged pereiopods, or damage to the tailfan and cephalothorax. One animal had a missing eye (although the stalk was present), and two animals had burn spot disease on the carapace.

Due to a very low incidence of freshly or recently moulted animals among the captures, it is difficult to determine a defined growing season for *Euastacus mirangudjin*. A high proportion of animals was recorded with a 'dirty' exoskeleton condition factor throughout the year (generally 35-80%, but lower in March during which no animals were captured) (see Figure 3). Only two of the five recaptured *Euastacus mirangudjin* had moulted between captures. In both instances, the crayfish displayed a moult increment of 1.5 mm (OCL). One male individual had moulted once, increasing from 32.3 to 33.8 mm OCL, between June and November (5 months). A female individual moulted from 28.3 to 29.8 mm OCL between December and January (1 month).

Reproductive biology

Size at Female Maturity

Female *Euastacus mirangudjin* achieve sexual maturity as they approach 30 mm OCL. Apart from one sexually mature animal just over 25 mm OCL, all sexually mature

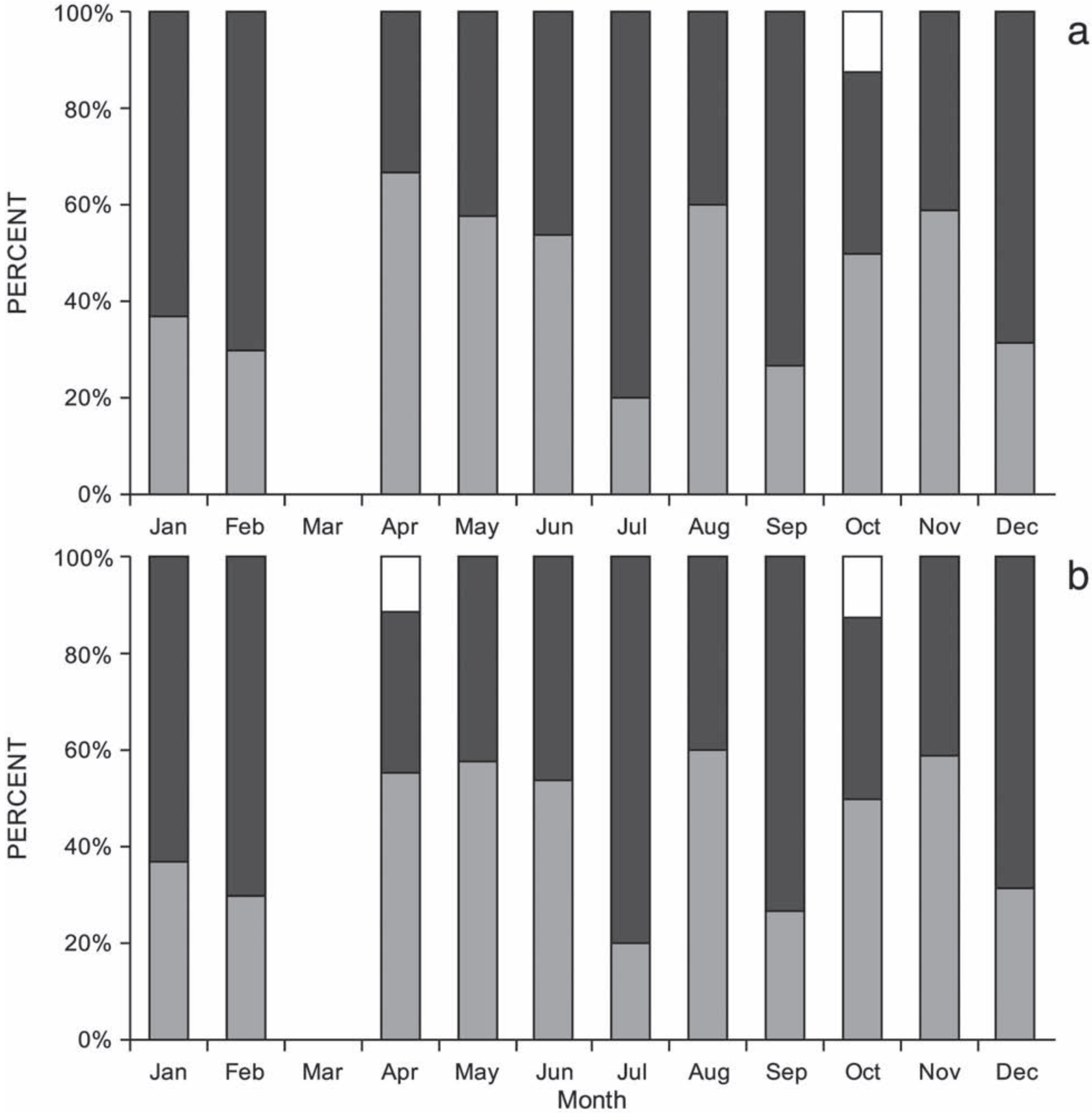

Figure 3. Exoskeleton condition factor for *Euastacus mirangudjin,* by month of capture, for: a) upper Iron Pot Ck (n=185); and b) all sites of record pooled (n=191). *Exoskeleton condition factor is indicated as follows: (i) grey – hard and clean; (ii) black – hard and dirty; (iii) white - very soft (fresh moult).* Data are restricted to animals >15 mm OCL.

females were close to 30 mm OCL (2 animals) or > 30 mm OCL (25 animals). All sexually immature females were < 30 mm OCL, and only one adolescent female was > 30 mm OCL (31.6 mm). The number of females with mature gonopore characteristics for 5 mm size classes is shown in Figure 4. The mean AbW/OCL ratio was 0.49 for both males and immature females (n=38), 0.51 for adolescent females (n=8) and 0.55 for mature females (n=28), confirming relative abdomen width as a useful secondary indicator of sexual maturity. The increasing relative abdomen width in mature females also results in significant sexual dimorphism in animals >30 mm OCL (t-test, $p < 0.0001$). Unlike other species, for which males were found to develop proportionally larger claws (Coughran 2006), there was no sexual dimorphism in relative propodal length for *E. mirangudjin* (t-test, $p > 0.5$).

Aberrant sexual characters

Six aberrant animals were encountered across all four species, representing approximately 3% of the population. Three of the aberrant specimens bore both male gonopores and a single female gonopore. Another animal bore one male and one female gonopore, each on different sides, and one animal bore two male and two abnormally developed female gonopores. Another animal bore only a single, abnormally developed female gonopore, and was carrying young when captured.

Breeding season and condition of reproductive females

Nineteen *Euastacus mirangudjin* females, ranging from 29.9-37.0 mm OCL, were carrying eggs or juveniles when captured. Of these, six had a regenerate cheliped, and another three had at least one cheliped missing (one

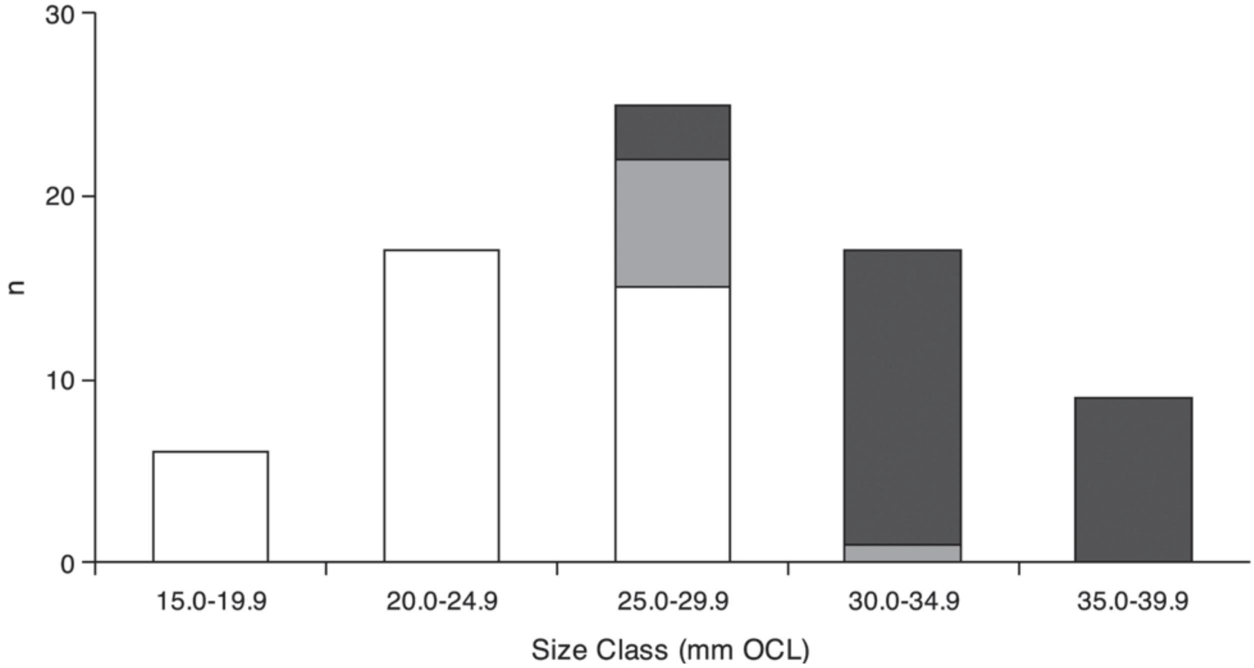

Figure 4. Number of mature females, by 5 mm size class, for female *Euastacus mirangudjin*. Based on gonopore states, females were classed as either immature (white), adolescent (grey), or mature (black). Data are presented for the long-term monitoring sites only.

female had both chelae, and the second left pereiopod, missing). With the exception of missing or regenerate chelipeds, no wounds or injuries were recorded for these reproductively active females. Although several of the females had clean exoskeleton condition states, most (11) had dirty exoskeleton conditions, suggesting that females do not routinely moult prior to spawning. Twelve of the nineteen reproductively active females carried temnocephalans. The timing of reproductive activity for *E. mirangudjin* females is shown in Figure 5. The data suggest that *E. mirangudjin* is an autumn-spring brooder, with release of juveniles in late spring. Most of the females above the minimum size at egg bearing that were captured during the reproductive season carried eggs or young, and fecundity ranged from 16-80 eggs/juveniles (mean 40.1). Fecundity generally increases with increasing female body size, with a distinct increase at around 36 mm OCL (close to the maximum size of 37 mm). Four large females over 36 mm OCL carried between 55-80 eggs or young, and females between 30-36 mm OCL carried 16-40 eggs.

Egg and juvenile development

Because of the very low number of eggs on the female *E. mirangudjin* retained for the egg development study, samples of eggs were taken for examination at less frequent intervals than for other species studied (Coughran 2006). As a result, the timing of the different developmental stages is uncertain for this species. Furthermore, documenting the full sequence of embryonic development was only possible by combining observations of eggs at different stages of development from

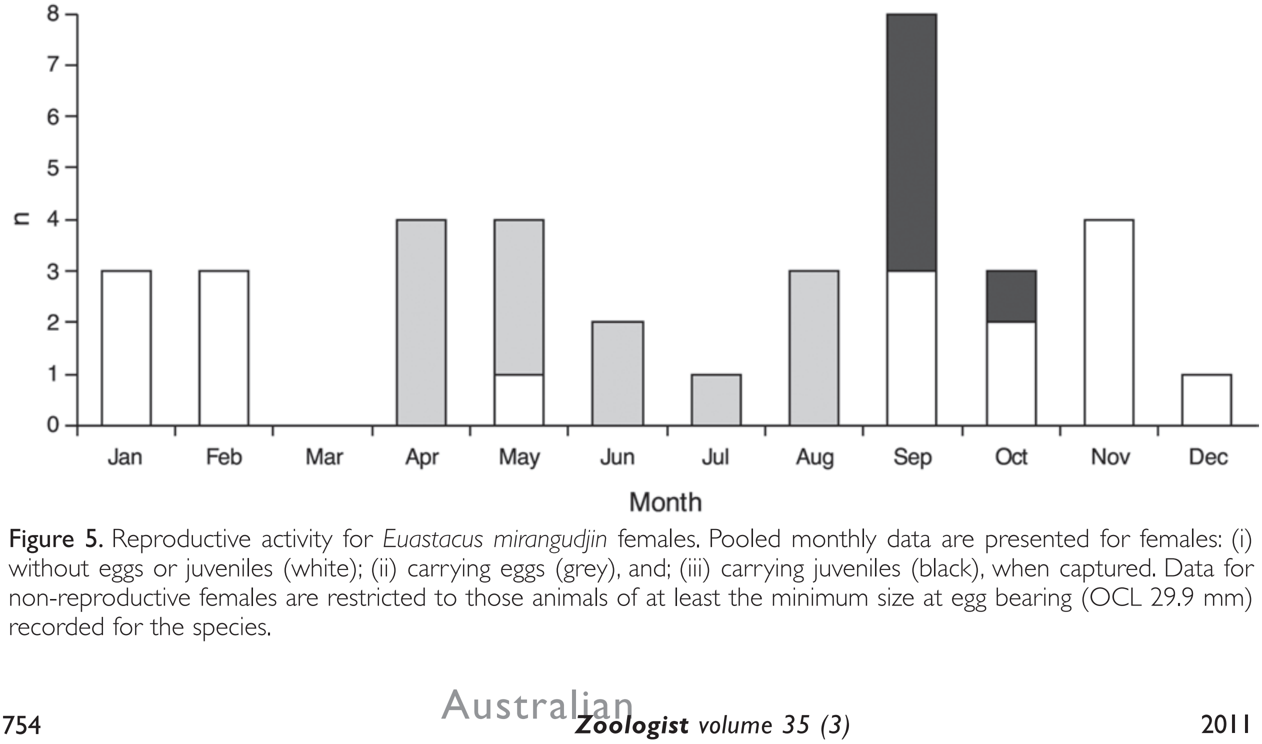

Figure 5. Reproductive activity for *Euastacus mirangudjin* females. Pooled monthly data are presented for females: (i) without eggs or juveniles (white); (ii) carrying eggs (grey), and; (iii) carrying juveniles (black), when captured. Data for non-reproductive females are restricted to those animals of at least the minimum size at egg bearing (OCL 29.9 mm) recorded for the species.

different females (i.e., no single female carried a sufficient number of eggs to allow for removal of samples over the entire duration of the development period). These eggs were also periodically examined under the microscope to observe developmental progress, and this information is used to supplement the observations based on the female carrying undeveloped (fresh) eggs at time of capture.

The eggs of *Euastacus mirangudjin* are ovoid in shape, opaque, pale tan or orange colour and around 3.0 mm x 3.8 mm. The eggs on one female did not become firmly cemented to the pleopodal setae, as recorded for the other species, and as a result many of the eggs were shed during the study period, even during late stages of development. After 46 days, no eggs remained on the female, as they had either been removed for examination or shed into the aquarium. However, juveniles were noticed in the dense leaf litter of the aquarium 111 days later, presumably having hatched from eggs shed during the course of the study. Although no laboratory retained *E. mirangudjin* carried juveniles after hatching, females were captured in the field studies with juveniles.

Discussion

Habitat and ecology

Euastacus mirangudjin is a small species of crayfish that is restricted to highland sites in subtropical rainforest. Although many other species of highland *Euastacus* have been noted to inhabit flowing streams (e.g. Morgan 1988, 1997), *E. mirangudjin* does not require sites with surface water or flow. It appears to be largely subterranean in its behaviour, spending most of its time within its burrow system. The species exhibits no response to meat baits, and despite repeated surveys (both diurnal and nocturnal) over two years only one animal was ever observed out of a burrow. This behaviour is quite different to that displayed by the larger *E. sulcatus*, a species that readily takes meat baits and is commonly observed walking in open water and even terrestrial habitats. Conceivably, the difference in behaviour of these two species may be partly attributed to the smaller size, and presumably a higher vulnerability to predators such as eels, of *E. mirangudjin*. To put this into perspective, *E. mirangudjin* only reaches around one third of the length, and less than one tenth of the weight, of *E. sulcatus* (Furse and Wild 2004; McCormack 2008). At some sites, no partitioning of the habitat between these two species is evident, and the two species can even be collected from beneath the same rock. But at Iron Pot Creek, there is a distinct partition between the two species: *E. mirangudjin* dominated the upper reaches, and *E. sulcatus* was restricted to lower elevations. Even at stream widths of over 5 m, very few *E. sulcatus* were recorded in the upper reaches of this site where *E. mirangudjin* occurred in abundance. Conversely, only occasional specimens of *E. mirangudjin* were recorded at the lower section of the site, where *E. sulcatus* was most abundant. Between the upper and lower sections there is an overlap zone, roughly coinciding with a long (>50 m) stretch with a solid basalt bedrock substratum, that appears to support few animals of either species. This type of co-occurrence of a smaller and larger species of *Euastacus* has been noted several times (see Coughran 2008).

The prevalence of injuries would suggest that there are regular interactions with other crayfishes (either conspecifics or the larger *Euastacus sulcatus*), or perhaps with other fauna. Around half of the *E. mirangudjin* animals examined in the study had at least one absent or regenerating cheliped. Additional wounds were recorded on 25% of animals, usually involving damaged, missing or regenerate walking legs. No notable signs of disease were recorded. A fish kill was observed shortly after logging activities upstream of the type locality and eels (*Anguilla reinhardtii*) were found dead or dying in and around the watercourse, with large ulcerations covering their bodies. However, no adverse effects were recorded for the crayfish. It is of note that *E. mirangudjin* was found to host only the one external species of Temnocephalan flatworm. Most other regional species of *Euastacus*, including the sympatric *E. sulcatus*, host two distinct external flatworms: a small, unpigmented species and a larger, pigmented species (Coughran 2006).

Growth and moulting

It is notable that *Euastacus mirangudjin* does not display any sexual dimorphism in male propodal length. Such sexual dimorphism has been widely documented for other crayfishes (Lindqvist and Lahti 1983; Sokol 1988; Guerra and Niño 1995; Hamr 1995, 1997; Honan and Mitchell 1995c; Morey 1998). Although the limited number of recaptures that had moulted was insufficient to derive growth estimates, the percent moult increment on the two animals that had moulted was similar to that observed for similar-sized *E. gumar* (Coughran 2011). Crayfish growth is a factor of both the moult increment and the intermoult period (Hartnoll 1983), and to provide more complete growth information for *E. mirangudjin* further research on both the intermoult period and moult increment is required.

Reproductive studies

Eggs were found to follow the general pattern of development observed for other parastacid crayfishes (Hopkins 1967; Hamr 1992; Honan 1998), the only notable difference being the failure of the eggs of *Euastacus mirangudjin* in the laboratory to cement onto the pleopodal setae of the female. It is uncertain if this is due to the laboratory conditions, or indicates that this is not a feature of the reproductive biology of this species. The latter alternative would be highly unusual, as cementing of eggs onto the setae is a well-documented aspect of the reproductive biology for both parastacoidean (Hopkins 1967; Suter 1977) and astacoidean (France 1983) crayfishes. France (1983) found acidification of the waterbody affected the glair cement on *Orconectes virilis*, resulting in severe recruitment failure. Although the laboratory conditions were close to neutral pH, it may be that successful cementing of eggs for *E. mirangudjin* requires a specific pH, or some other aspect of water chemistry. Observations in the field for *E. mirangudjin* suggest that there are also three intermoult stages of juveniles prior to independence from the mother, although this could not be verified during laboratory studies due to the loss of uncemented eggs from the clutch. Limited visual observations in the laboratory

and field found no juveniles with the characteristic vivid cream abdominal bands that occur on juveniles of other *Euastacus* species (see Coughran 2008). *Euastacus mirangudjin* carries fewer eggs than other regional species, *E. gumar*, *E. sulcatus* and *E. valentulus*, but the eggs are relatively larger (Coughran 2006; Coughran 2011). The low fecundity of *E. mirangudjin* does not lend itself to detailed developmental studies of eggs from a single clutch. Further studies on egg and juvenile development should attempt to examine egg development without removal from the clutch.

The knowledge of the biology and ecology of this rare species remains limited. Collection of data for sub-surface species such as this is labour intensive, and increasing our understanding of the species further will require considerable effort and funding. Future research efforts may initially be most productive if focused around the breeding season (Apr-Oct). Given the difficulty in capturing specimens, and the conservation concerns for the species, future research may need to focus on the species within its burrow system, trialing novel techniques (i.e. fibre optic cameras).

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